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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEWER S-TRIAZINE DERIVATIVES

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ABSTRACT

A variety of 4, 6-dichloro-N-(3-substituted phenyl)-1, 3, 5-triazin-2-amine derivatives (B1 to B6) and 4, 6-dichloro-N-(4-substituted phenyl)-1, 3, 5-triazin-2-amine derivatives (C1 to C6) were prepared by reacting cyanuric chloride with amine, substituted acetophenone and bromination of the ketone group followed by cyclization using urea/ thio urea/ thio semicarbazole/ amino guanidine. HCl/ acetamide/ benzamide to prepared the respective compounds. The structures of all the compounds were confirmed by spectral analysis. The newly synthesized compounds were evaluated for antimicrobial activity against a variety of bacterial strains and fungal strains and some of these compounds have shown significant antibacterial and antifungal activities.

Keywords: 1, 3 5- triazine, cyanuric chloride, antimicrobial activity.

INTRODUCTION

Antibacterial and antifungal diseases are very common all over the world. Currently used antimicrobial agents are not effective due to the resistance developed by the microbes. And therefore, it is an ongoing effort to synthesize new antimicrobial agents. Over and above there is no permanent structure and activity relationship [1].

1,3,5-triazine core, possessing three-fold symmetry, allows for versatile modifications, uncomplicated by regiochemical concerns and has proven a useful biological target [2]. The structural modification of these heterocycles at the 2-, 4-, and 6- positions has led to the development of several derivatives and associated with a wide range of therapeutic activities such as antibacterial[3], fungicidal [3], anticancer [4], antitubulin [5], antitrypanosomal [6], anti leishmanial [7], anti-inflammatory [8], thymidine phosphorylase inhibitors [9], anti-malarial [10], anti tmv [11], phosphorescent organic light emitting devices [12], electron transport-type host materials for highly efficient green phosphorescent OLEDs [13]. In addition, compounds possessing s-triazine core are also found to have activities [3] like antiretroviral, antiviral, antiulcer, antiarthritic, local anesthetic, anticonvulsant, algaecide and disinfectant, hypoglycemic, analgesic, sedative, anthelmintic and antitubercular.

To randomly explore the novel compounds, our idea was to combine cyanuric chloride with amine substituted acetophenone and bromination of the ketone group followed by cyclization with urea/ thiourea/ thio-semicarbazole/ amino guanidine. HCl/ acetamide/ benzamide to synthesized the respective derivative compounds. Substituted s-triazine derivatives remain attractive, with their significant biological activities [3].

MATERIALS AND METHODS

All the melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked routinely by TLC using silica gel coated plates and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (υ_{max} in cm^{-1}) were recorded on FT-IR-Spercle Elmer DHF1FT-IR using KBr technique. The ^{1}HNMR and ^{13}C NMR spectra of the compounds were carried out in Bruker AMX 400 MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The mass spectra of the compounds were carried out in Agilent 1100 series LC-MSD.

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Synthesis of 4,6- dichloro-N- (3 or 4- substituted phenyl)- 1,3,5-triazin-2-amine 1-(3 or 4 substituted-(4,6-dichloro-1,3,5-triazin-2-ylamino) phenyl) ethanone (1)

Amine substituted acetophenone (0.01 mole) was added slowly to cyanuric chloride (0.01 mole) in acetone (30 ml) with constant stirring over a period of 4 hr at 0 to 5^0 C. Then, sodium carbonate (0.005 mole) dissolved in water (10 ml) was added drop wise to neutralize HCl evolved during the reaction. Finally, the contents were poured into crushed ice. The solid was separated out by filtration and washed with water. The product is dried, recrystallized from alcohol to give the product (1).

Synthesis of 1-(3 or 4 substituted-(4,6-dichloro-1,3,5-triazin-2-ylamino)phenyl)-2-bromoethanone (2)

To a solution of compound **1** (0.095 mole) in 150 ml of glacial acetic acid, bromine (0.1 mole) in 20 ml glacial acetic acid was added with stirring for 0.5 hr at room temperature. The mixture was then warmed to decompose an addition product. The mixture was heated for 15 min on a water bath to expel most of the hydrogen bromide, cooled and filtered. It was then poured into ice cold water and the solid separated out was filtered, washed with water and dried. The product was washed with ether and recrystallised from alcohol.

Synthesis of 4,6- dichloro- N- (3 or 4-substituted phenyl)-1,3,5- triazin-2-amine (3)

A suspension of compound **2** (0.056 mole) in 15 ml of hot ethanol was treated with urea/ thio urea/ thio semicarbazole/ amino guanidine. HCl/ acetamide/ benzamide (0.06 mol) a mild exothermic reaction took place, gave a clear solution that soon deposited as crystals. The deposit was removed, washed with ethanol and then boiled with water containing sodium acetate which yielded the derivative compound 3 (B1 to B6 and C1 to C6). The product was recrystallised with absolute ethanol. The characterization data of the compounds is shown in Table No.1.

Spectral data of the synthesized derivative compounds

B1: IR (in KBr) υ, cm^{-1}: 1083.73 (Ar C –Cl, ;Str), 1159.90 (secondary N –C ;Str), 1293.75 (Ar N ;Str), 1531.95 (primary N –H ;Bend), 3301.86 (secondary N –H ;Str), 3399.45 (primary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 4.47 (d, 2H, -CH_2), 5.10 (t, 1H, CH), 5.63 (s, 3H, -NH, -NH_2), 7.71 (d, 1H, Ar- H), 7.79 (t, 1H, –CH), 8.2 (d, 1H, Ar –H), 8.32 (s, 1H, Ar –H)

C^{13}NMR: (δ in DMSO, ppm): 65.26 (^{11}C), 66.36 (^{10}C), 120.18 (^{7}C), 124.30 (^{8}C), 125.04 (^{4}C), 129.50 (^{5}C), 137.51 (^{6}C), 152.65 (^{9}C), 163.93 (^{12}C), 169.84 (^{1,3}C), 171.92 (^{2}C)

B2: IR (in KBr) υ, cm^{-1}: 1044.67 (Ar C –Cl, ;Str), 1172.49 (secondary N –C ;Str), 1339.34 (Ar N ;Str), 1505.91 (primary N –H ;Bend), 3285.19 (secondary N –H ;Str), 3771.91 (primary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 3.32 (d, 2H, -CH_2), 5.10 (t, 1H, -CH), 5.53 (s, 3H, -NH), 7.32- 7.41 (m, 2H, Ar –H), 7.51- 7.93 (m, 2H, Ar –H)

B3: IR (in KBr) υ, cm^{-1}: 1018.61 (Ar C –Cl, ;Str), 1217.78 (secondary N –C ;Str), 1308.08 (Ar N ;Str), 1535.71 (Aliphatic primary N –H ;Bend), 3061.12 (secondary N –H ;Str)

^{1}HNMR (CDC13) δ, ppm: 3.31 (d, 2H, -CH_2), 4.83- 4.86 (t, 1H, –CH), 6.01 (s, 4H, -NH), 7.26- 7.65 (m, 4H, Ar –H)

B4: IR (in KBr) υ, cm^{-1}: 1015.04 (Ar C –Cl, ;Str), 1160.06 (C –O –C ;Asymm. str), 1218.65 (secondary N –C ;Str), 1296.34 (Ar N ;Str), 3303.24 (secondary N –H ;Str)

^{1}HNMR (CDC13) δ, ppm: 2.64 (s, 3H, -CH_3), 4.2 (d, 2H, -CH_2), 5.23 (t, 1H, -CH), 7.55- 7.65 (m, 2H, Ar-H), 7.79- 7.87 (m, 2H, Ar –H)

B5: IR (in KBr) υ, cm^{-1}: 1014.54 (Ar C –Cl, ;Str), 1159.54 (C –O –C ;Asymm. str), 1219.03 (secondary N –C ;Str), 1295.90 (Ar N ;Str), 3302.14 (secondary N –H ;Str)

^{1}HNMR (CDC13) δ, ppm: 4.47 (d, 2H, -CH_2), 5.24 (t, 1H, -CH), 7.51- 7.58 (m, 5H, Ar –H), 7.66- 7.68 (m, 2H, Ar –H), 7.79- 7.87 (m, 2H, Ar –H)

MS: m/z 385 (M –)

B6: IR (in KBr) υ, cm^{-1}: 1010.86 (Ar C –Cl, ;Str), 1223.75 (secondary N –C ;Str), 1322.32 (Ar N ;Str), 1549.62 (primary N –H ;Bend), 3267.92 (secondary N –H ;Str), 3775.21 (primary N –H ;Str)

^{1}HNMR (MeOD) δ, ppm: 4.21 (d, 2H, -CH_2), 5.10- 5.27 (t, 1H, -CH), 5.63 (s, 5H, –NH), 7.40- 7.75 (m, 2H, Ar –H), 7.79 (s, 2H, Ar –H)

C1: IR (in KBr) υ, cm^{-1}: 1062.19 (Ar C –Cl, ;Str), 1182.60 (secondary N –C ;Str), 1322.03 (Ar N ;Str), 1548.98 (primary N –H ;Bend), 3298.90 (primary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 4.20 (d, 2H, -CH_2), 5.10-5.16 (t, 1H, -CH), 5.42 (s, 3H, –NH), 7.72- 7.84 (m, 4H, Ar –H)

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C2: IR (in KBr) υ, cm^{-1}: 1063.29 (Ar C –Cl, ;Str), 1184.76 (secondary N –C ;Str), 1326.32 (Ar N ;Str), 1554.87 (primary N –H ;Bend), 3298.81 (secondary N –H ;Str), 3763.57 (primary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 3.76 (d, 2H, -CH_2), 4.76 (s, 2H, –NH), 6.08 (t, 1H, -CH), 7.75- 7.84 (m, 4H, Ar –H)

C3: IR (in KBr) υ, cm^{-1}: 1012.71 (Ar C –Cl, ;Str), 1224.75 (secondary N –C ;Str), 1323.12 (Ar N ;Str), 1550.16 (primary N –H ;Bend), 3137.23 (primary N –H ;Str), 3266.91 (secondary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 3.42 (d, 2H, -CH_2), 4.47-4.52 (t, 1H, –CH), 5.15 (s, 4H, -NH), 7.76- 8.13 (m, 4H, Ar –H)

C4: IR (in KBr) υ, cm^{-1}: 1011.76 (Ar C –Cl, ;Str), 1165.30 (secondary N –C ;Str), 1322.08 (Ar N ;Str), 1384.37 (-CH_3 ;Symm. bend), 1425.40 (-CH_3 ;Asymm. bend), 3268.07 (secondary N –H ;Str)

^{1}HNMR (CDC13) δ, ppm: 2.0 (s, 3H, -CH_3), 4.01 (d, 2H, -CH_2), 5.30 (t, 1H, -CH), 6.63 (s, 1H, -NH), 7.68-8.19 (m, 4H, Ar –H)

C5: IR (in KBr) υ, cm^{-1}: 1011.53 (Ar C –Cl ;Str), 1184.84 (secondary N –C ;Str), 1322.80 (Ar N ;Str), 3268.29 (secondary N –H ;Str)

^{1}HNMR (in DMSO d_6) δ, ppm: 4.22 (d, 2H, –CH_2), 5.14 (t, 1H, –CH), 7.72- 8.13 (m, 9H, Ar-H)

C6: IR (in KBr) υ, cm^{-1}: 1010.86 (Ar C –Cl ;Str), 1223.75 (secondary N –C ;Str), 1322.32 (Ar N ;Str), 1549.62 (Aliphatic primary N –H ;Bend), 3267.92 (secondary N –H ;Str), 3775.21 (primary N –H ;Str)

^{1}HNMR (CDC13) δ, ppm: 3.50 (d, 2H, -CH_2)**,** 5.50 (t, 1H, –CH), 6.63 (s, 5H, –NH), 7.68- 8.19 (m, 4H, Ar-H)

MS: m/z 340 (M +)

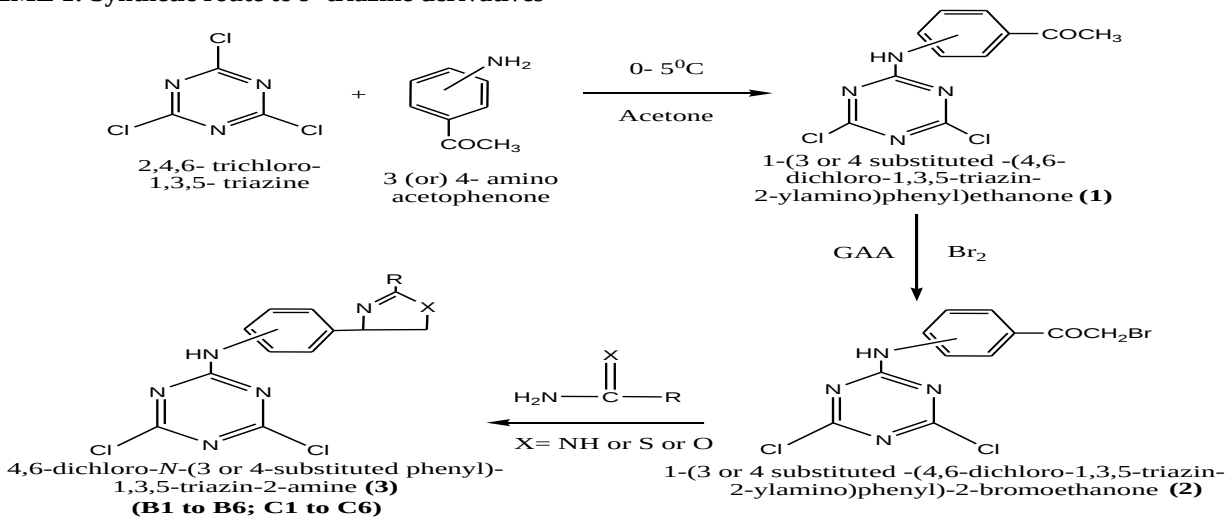
Antibacterial activity

The *invitro* antibacterial screening of all the compounds (B1 to B6 and C1 to C6) were evaluated against Gram- positive organisms *Staphylococcus aureus* (MTCC 96), *Bacillus pimilis* (NCIM 2063) and Gram-negative organism *Escherichia coli* (MTCC 443) by cup and plate method[14]. The culture was maintained on nutrient agar slants. Twenty milliliters of sterilized nutrient agar medium was inoculated with the bacteria and spread in a petridish and allowed to set for 30 min. Four bores (10 mm in diameter) were made at equal distance in the petridish and filled with a single standard concentration (50 µg/ml) of standard drug Streptomycin and different concentrations (50 and 100 µg/ml) of triazine derivatives were introduced. Dimethyl formamide was used as a control. After introduction of standard drug and extracts, the plates were placed in a refrigerator at 8-100C for proper diffusion into media. After two hrs of cold incubation, the petriplates are transferred to incubator and maintained at 370 for 24 hrs. After the incubation period, the petriplates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zones of inhibition shown by the derivatives with standard drug.

Antifungal activity

Aspergillus niger and Penicillium notatum were employed for testing antifungal activity using the cup-plate method [14]. Miconazole is used as standard and DMSO is used as control.

SCHEME 1: Synthetic route to s- triazine derivatives



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RESULTS AND DISCUSSION

Antibacterial and antifungal diseases are very common all over the world. Currently used antimicrobial agents are not effective due to the resistance developed by the microbes. And therefore, it is an ongoing effort to synthesize new antimicrobial agents. *In vitro* antibacterial activity data of s-triazine derivatives against tested organisms displayed significant activity. The compounds B1, B3, B6, C3, C4 and C5 were found to have remarkable activity against *gram +ve* bacteria, *S. aureus;* B3, B4, B6, C2, C3, C4, C5 and C6 were found to have very good activity against *gram +ve* bacteria *B. pimilis*, and B1, B2, B3, B6, C1, C3, C5 and C6 were found to be very active against *gram –ve E. coli*. The other compounds were found to possess moderate to low inhibition on gram *+ve* and *gram –ve* bacteria**.** All the compounds were compared to the standard Streptomycin. The zones of inhibition of the compounds on the bacteria are shown in the Table No.2 & Figure 1.

B1 and C6 were found to show remarkable activity against both *Aspergillus niger* and *Pencillium notatum.* C2 and C3 were found to have good activity against *P.notatum*. B2 and B3 were found to possess moderate activity against *P.notatum.* Remaining compounds were found to show minimal activity against fungi**.** All the compounds were compared with standard miconazole nitrate. The zones of inhibition of the compounds on the fungi are shown in the table no.3.

Table 1. Characterization data of the synthesized derivative compounds

Compound code	X	R	Mol. formula	Mol. Weight (g/ mole)	Melting point (^{0}C)	% yield	R_f value*
B1	O	NH_2	$C_{12}H_{10}Cl_2N_6O$	325.15	170-178	49.32	0.53
B2	S	NH_2	$C_{12}H_{10}Cl_2N_6S$	341.22	260-264	17.25	0.55
B3	S	$NHNH_2$	$C_{12}H_{11}Cl_2N_7S$	356.23	180-183	54.16	0.46
B4	O	CH_3	$C_{13}H_{11}Cl_2N_5O$	324.17	164-170	54.54	0.57
B5	O	C_6H_5	$C_{18}H_{13}Cl_2N_5O$	386.23	168-170	37.03	0.59
B6	NH	$NHNH_2$	$C_{12}H_{12}Cl_2N_8$	339.18	166-168	46.95	0.50
C1	O	NH_2	$C_{12}H_{10}Cl_2N_6O$	325.15	240-242	46.94	0.60
C2	S	NH_2	$C_{12}H_{10}Cl_2N_6S$	341.22	180-185	45.58	0.56
C3	S	$NHNH_2$	$C_{12}H_{11}Cl_2N_7S$	356.23	248-250	48.34	0.90
C4	O	CH_3	$C_{13}H_{11}Cl_2N_5O$	324.17	255-258	38.46	0.55
C5	O	C_6H_5	$C_{18}H_{13}Cl_2N_5O$	386.23	280-284	23.52	0.61
C6	NH	$NHNH_2$	$C_{12}H_{12}Cl_2N_8$	339.18	292-294	18.66	0.54

*mobile phase = Ethylacetate: methanol (8:2)

Table 2. Zone of inhibition (in mm) obtained on bacteria

S.No	Compound code	Gm *+ive*				Gm *-ive*	
		S.aureus		***B. Pimilis***		***E.Coli***	
		50 µg/ml	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**
1	B1	13	16*	10	11	13	16*
2	B2	10	12	10	11	10	15*
3	B3	10	11	14*	20*	20*	21*
4	B4	12	13	13	17*	12	13
5	B5	12	13	12	13	12	13
6	B6	12	13	16*	20*	15*	23*
7	C1	12	13	11	13	14*	15*
8	C2	11	12	14*	19*	12	13
9	C3	13	17*	13	19*	16*	21*
10	C4	15*	18*	14*	17*	12	13
11	C5	13	19*	14*	19*	15*	17*
12	C6	12	13	14	18*	15*	20*
Control	DMF	9		9		9	
Standard	Streptomycin (100 µg/ml)	23*		20*		24*	

(*) Significant zone of inhibition bore size- 10 mm

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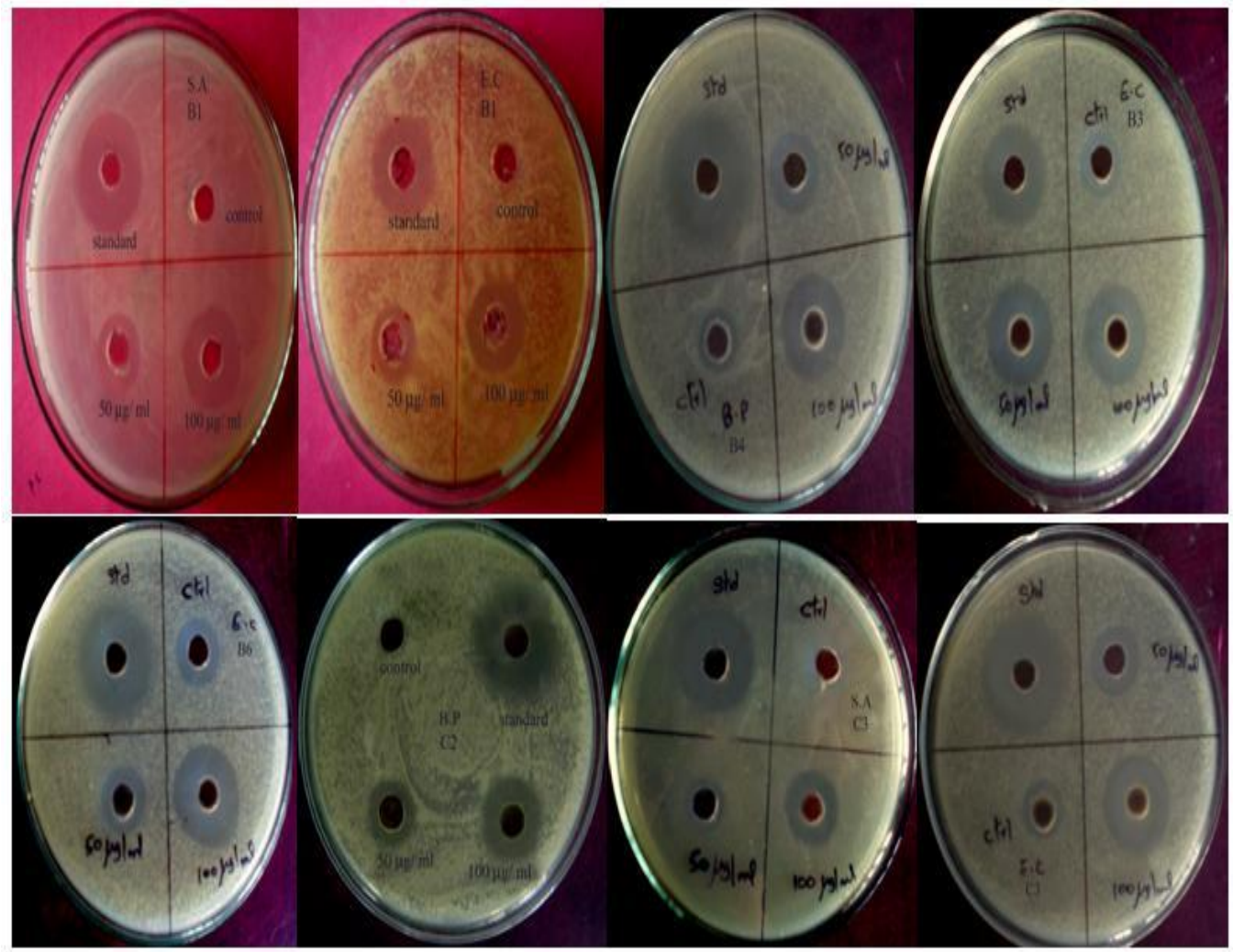
Table 3. Zone of inhibition (in mm) obtained on fungi

S.NO	COMPOUND CODE	*Aspergillus niger*		*Penicillum notatum*	
		50 µg/ml	100 µg/ml	50 µg/ml	100µg/ml
1	B1	14	16*	15*	20*
2	B2	12	13	15*	16*
3	B3	12	14	13	15*
4	B4	12	13	11	13
5	B5	11	12	12	14
6	B6	12	14	10	13
7	C1	13	14	11	13
8	C2	12	13	14	16*
9	C3	13	14	16*	20*
10	C4	11	13	10	12
11	C5	12	13	12	13
12	C6	14	17*	14	18*
Control	DMF	10	10	10	10
Standard	MICONAZOLE NITRATE(50 µg/ml)	25*		20*	

(*) Significant zone of inhibition bore size- 10 mm

Figure 1. *In vitro* antibacterial activity data of s-triazine derivatives against tested organisms



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CONCLUSION

Substituted s-triazine derivatives, B1 to B6 and C1 to C6 were synthesized and characterized for their structure elucidation. Antibacterial and antifungal studies of these compounds indicated that compounds were found to be showing comparable activity against some bacteria compared to standard antibiotic drugs.

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